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# Spontaneous vegetation colonizing abandoned metal(loid) mine tailings consistently modulates climatic, chemical and biological soil conditions throughout seasons

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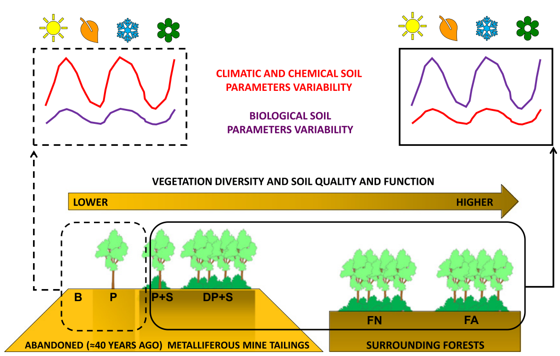
## HIGHLIGHTS

- Mine tailings soils improvement by spontaneous vegetation was seasonally consistent.
- Bare soils showed high seasonal variability in climatic and chemical conditions.
- Plant patches soften the harsh climatic conditions of summer and winter like forests.
- Plant patches showed stronger changes in soil biological parameters like forests.
- Interest in preserving spontaneous plant patches for mine tailings phytomanagement.

## GRAPHICAL ABSTRACT

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## ABSTRACT

This study aimed to evaluate whether the improvement in soil conditions induced by the vegetation spontaneously colonizing abandoned metal(loid) mine tailings from semiarid areas is consistent throughout seasons and to identify if the temporal variability of that conditions is of similar magnitude of that of the surrounding forests. Soil climatic (temperature and moisture), chemical (pH, electrical conductivity and water-soluble salts and metal(loid)s) and biological (water-soluble organic carbon and ammonium, microbial biomass carbon, dehydrogenase and β-glucosidase activity, organic matter decomposition and feeding activity of soil dwelling organisms) parameters were seasonally evaluated for one year in bare soils and different vegetated patches within metalliferous mine tailings and surrounding forests in southeast Spain. The results indicated that the improvement in soil conditions (as shown by softening of climatic conditions and lower scores for salinity and water-soluble metals and higher for biological parameters) induced by vegetation colonization was consistent throughout seasons. This amelioration was more evident in the more complex vegetation patches (trees with herbs and shrubs under the canopy), compared to bare soils and simpler soil-plant systems (only trees), and closer to forest soils outside the tailings. Bare soils and, to a lesser extent, vegetation patches solely composed by trees, showed stronger seasonal variability in temperature, moisture content, salinity, and water-soluble metals. In contrast, changes in biological and biological-related parameters were more pronounced in the more complex vegetation patches within mine tailings and surrounding forests due to its greater biological activity. In summary, the results demonstrated that vegetation patches formed by spontaneous colonization act as microsites that modulate seasonal variability in soil conditions and stimulate biological activity. This suggests that tailings vegetation patches might have higher resilience against climate change effects than bare soils. Therefore, they should be preserved as valuable spots in the phytomanagement of metal(loid)s mine tailings from semiarid areas.

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## 1. Introduction

Mine tailings from sulfide mining areas are among the most hostile environments for biota worldwide (Niemeyer et al., 2012; Brown et al., 2014; Navarro-Cano et al., 2018). Tailing wastes are usually highly saline, lack of organic matter (quantity and quality) and nutrients and undergo severe compaction and/or crusting processes (Wong, 2003; Mendez and Maier, 2008). But, due to the high metal(loid) levels (*i.e.*, potentially toxic elements, PTEs), their major drawback is that they pose a serious risk to environmental and human health. When tailings are abandoned without any restoration measure, their surface is exposed to the atmosphere and the materials stored suffer chemical (*e.g.*, oxidation of sulfides and acidification) and physical (*e.g.*, erosion) alteration. This contributes to the spread of dissolved and/or particulate PTEs to the surroundings (Nordstrom, 2011; Nordstrom et al., 2015).

Phytomanagement by phytostabilization is one of the most promising strategies to mitigate mine tailings impacts (Mendez and Maier, 2008; Antoniadis et al., 2017; Burges et al., 2018). It involves the establishment of a permanent vegetation cover on the contaminated site to reduce PTEs transfer and improve the value of the land (Robinson et al., 2009). Phytostabilization also seeks to restore ecosystem functionality (Burges et al., 2018) and the ecological integration of abandoned mining structures within the landscape matrix (Tordoff et al., 2000; Mendez and Maier, 2008; Navarro-Cano et al., 2018). In line with this, several authors have pointed out that phytomanagement may take advantage from knowing the processes involved in the spontaneous colonization of mine tailings by biota (*i.e.*, passive restoration) (Prach and Hobbs, 2008; Gutiérrez et al., 2016; Prach and Tolvanen, 2016), including (micro)organisms and vegetation (*e.g.*, Navarro-Cano et al., 2018; Colin et al., 2019; Risueño et al., 2020). Plant species adapted to local climate and stressful soil conditions may colonize tailings surface by forming vegetated patches in which ecological linkages between (micro)organisms and aboveground plant communities occur (Huang et al., 2012). The formation of these so-called "fertility islands" has been extensively studied around the world in a variety of ecosystems such as African savannas (Ludwig et al., 2004; Muvengwi et al., 2015), Australian mulga land (Tongway and Ludwig, 1996), continental forest-steppes (Tölgyesi et al., 2018), semiarid shrublands (Barbosa-Briones et al., 2019), Mediterranean semiarid steppes (Maestre and Cortina, 2002) and desert-oasis ecotones (Wang et al., 2019a). In general, soils beneath plant canopy are more fertile, with higher microbial activity and milder climatic conditions than in adjacent gaps (Wang et al., 2019a, 2019b). This is of particular importance in abandoned mine tailings that are considered barren substrates without biological legacy (Colin et al., 2019). In these environments, compared to bare areas, vegetated patches' soils have shown milder microclimatic conditions (Oreja et al., 2020), lower salinity and metal solubility, development of pedogenetic processes and similar functionality to that of nearby forests (Álvarez-Rogel et al., 2021; Peñalver-Alcalá et al., 2021). Therefore, tailings vegetated patches host more functional soils than gaps between them, and higher functionality is expected to confer greater resilience against the effects of climate change (Kardol et al., 2010; Lal, 2012).

The studies performed in abandoned mine tailings mentioned above were mostly carried out at punctual periods of the year. However, there is a lack of information on the seasonal behavior of soils under vegetated tailings patches. This might be a key aspect for the maintenance of ecosystem functionality, particularly in drylands where sharp variations in climate conditions occur throughout the year (Jia et al., 2020). Furthermore, this issue might be critical in the context of the current climate change perspective since increasing temperatures are expected to alter soil biological activity, especially in nutrient-limited systems (Zuccarini et al., 2020). Analyzing seasonal changes in a set of soil parameters that are usually measured to follow the outcome of restoration actions in metal(loid)-polluted sites might contribute to a more accurate assessment of the value that spontaneously vegetated patches have for mine tailings phytomanagement. This set may include soil temperature and moisture (e.g., Oreja et al., 2020), pH, salinity and metal(loid) availability (*e.g.*, Párraga-Aguado et al., 2013; Parra et al., 2016; García-Carmona et al., 2017; Mourinha et al., 2022), and the activity of enzymes related to organic matter and nutrients cycling (*e.g.*, Párraga-Aguado et al., 2014; Navarro-Cano et al., 2015, 2018; Risueño et al., 2020).

The present study aimed to assess if the improvement in soil conditions induced by the vegetation spontaneously colonizing abandoned metal(loid) mine tailings from semiarid areas is consistent throughout seasons and to identify if the temporal variability of that conditions is of similar magnitude of that of the surrounding forests. For this, a set of climatic, chemical, and biological parameters were seasonally evaluated for one year, under laboratory and in situ field conditions, in metal(loid) mine tailings soils and surrounding forests from an abandoned mining area in SE Spain. We initially hypothesized that the beneficial effects of vegetation on the soils beneath would be consistent, but modulated, over the seasons, and that the changes would follow a similar pattern to that of the forest soils outside the mine tailings.

## 2. Materials and methods

### 2.1. Study area and environments

The study was carried out in the former metal mining district of La Unión-Sierra de Cartagena located in SE Spain (Cartagena, Murcia Region; 37°37′20″N, 00°50′55″W-37°40′03″N, 00°48′12″W; Fig. S1, Suppl. Mat.). It is a Mediterranean semiarid area (average annual temperature ~ 17 °C, average annual precipitation ~200–300 mm and average annual evapotranspiration rate ~ 850 mm). The territory was exploited for metal ores since Roman times, but the impacts became more intense during the second half of the 20th century due to the use of modern intensive techniques (Conesa and Schulin, 2010). Iron is present as oxides, hydroxides, sulfates, sulfides, carbonates, and silicates; zinc and lead as sulfides, sulfates, carbonates, and zinc- or lead-bearing (Mn, Fe) oxides (Conesa and Schulin, 2010). When the activity stopped in 1991, 89 mine tailings storing 23 $Mm^3$ of mine wastes were abandoned in situ without any restoration measure. These tailings occupy ~2.18 $km^2$, have an average height of ~20 m and a surface of ~40,000–80,000 $m^2$. They present high risk of dispersion of PTEs by leaching and water and/or wind erosion (Conesa and Schulin, 2010; Robles-Arenas and Candela, 2010; Sánchez-Bisquert et al., 2017; Blondet et al., 2005). Most of the tailing surfaces remain bare (Conesa and Schulin, 2010; Pellegrini et al., 2016), but ~20–30% have been spontaneously colonized by the surrounding native vegetation, shaping a patchy structure.

The environments under study consisted of patches of spontaneous vegetation and a bare area located inside two mine tailings built by mid-60s to store wastes from galena ore mines (IGME, 2002) and that were abandoned ~40 years ago, and the surrounding forests (Fig. S1, Suppl. Mat.). Both tailings are embedded in small valleys NW to SE facing, at a similar altitude (~170–200 m a.s.l.) and located less than ~2000 m away of each other. Four environments were studied inside and two outside the mine tailings. Inside the tailings (Fig. S2, Suppl. Mat.): Bare soils (B); Small groups of *Pinus halepensis* trees <~2.5 m high growing scattered (P); Isolated *P. halepensis* trees >~4 m high with shrubs and herbs under the canopy (P + S); Dense patches including several *P. halepensis* trees >~4 m high and shrubs and herbs under the canopy (DP + S). Outside the tailings (Fig. S3, Suppl. Mat.): Forest located next to the tailings, with *P. halepensis* trees >~5 m high and shrubs and herbs under the canopy (FN); Forest located away from the tailings (~1600–1800 m), with *P. halepensis* trees >~5 m high and shrubs and herbs under the canopy (FA). The pine tree *P. halepensis* was the only plant species common to all the study environments (except in B). Among the species growing beneath pine trees, the perennial grasses *Brachypodium retusum* and *Lygeum spartum* appeared in P + S, DP + S and FN, *Piptatherum miliaceum* in P + S and DP + S, and *Stipa tenacissima* in FA. The dwarf shrubs *Helianthemum syriacum* and *Helichrysum decumbens* were present in DP + S; the shrubs *Pistacea lentiscus* in P + S, DP + S and FN, *Rhamnus lycioides* in DP + S and FN, and *Calicotome intermedia* in FA. The Margalef diversity index was significantly lower in P (~0.8), without differences among the other vegetated environments (~1.8–2.6) (Álvarez-Rogel et al., 2021; Peñalver-Alcalá et al., 2021).

In each environment four plots (2 m × 2 m) were established to study the soil conditions throughout a year, from June 2017 to June 2018. Composite soil samples (upper ~15 cm) were collected from each plot in June 2017 for a general characterisation ($n = 4$ per environment) (Table S1, Suppl. Mat.). The soils were sandy textured, with sand contents ~60–85%. Total $CaCO_3$ contents (in g $kg^{-1}$) were < 1 (B), ~30–55 (P, P + S and DP + S) and ~ 90–150 (FN and FA); cation exchange capacity (CEC, in $cmol_c$ $kg^{-1}$) ~5–13 within the tailings and ~ 20–30 in the forests; total organic carbon (TOC, in g $kg^{-1}$) ~3–12 within the tailings and ~ 40–100 in the forests; and total nitrogen (TN, in g $kg^{-1}$) ~0.4–0.7 within the tailings and ~ 2–4.5 in the forests. Total PTE concentrations tended to be higher within mine tailings, but a consistent variation pattern among study environments was not observed (Table S1, Suppl. Mat.). Total Fe (T-Fe) was ~2.7 g 100 $g^{-1}$ in FA and ~ 15.3–29.2 g 100 $g^{-1}$ in the other environments; total As (T-As) was ~70–250 mg $kg^{-1}$ in FA, B and P, and ~ 620–1250 mg $kg^{-1}$ in P + S, DP + S and FN; total Cd (T-Cd) ranged between <10 and ~ 55 mg $kg^{-1}$; total Cu (T-Cu) was ~49 mg $kg^{-1}$ in FA and ~ 142–277 mg $kg^{-1}$ in the other environments; total Mn (T-Mn) was ~1500–1800 mg $kg^{-1}$ in B and FA and ~ 7500–10,800 mg $kg^{-1}$ in the other environments; total Pb (T-Pb) varied from ~1300 mg $kg^{-1}$ in FA to ~14,500 mg $kg^{-1}$ in P + S; total Zn (T-Zn) was ~760 mg $kg^{-1}$ in FA and ~ 9000–18,000 mg $kg^{-1}$ in the other environments.

### 2.2. Seasonal soil sampling and laboratory analyses

Four periods were considered for monitoring: from June to August 2017 (summer), from October to the beginning of December 2017 (autumn), from the end of December 2017 to the beginning of March 2018 (winter), and from the end of March to June 2018 (spring). One composite soil sample (upper ~15 cm) was seasonally collected within each plot ($n = 4$ per environment) and taken to the laboratory inside refrigerated bags. Soil moisture content was evaluated in aliquots weighted before and after drying at 70 °C until constant weight ($n = 2$ per plot of study environment). The remaining material was stored at −20 °C and thawed at room temperature prior to performing the analyses described below.

Aliquots of thawed samples were used to perform water extractions (1:2.5 soil:water suspensions; 2 h shaking, rotatory shaker). The required water volume was adjusted based on the field soil moisture content. Immediately after shaking, water extracts were filtered through nylon membrane syringe filters (0.45 μm, WICOM) and pH and electrical conductivity (EC) measured (Crison Basic 20 pH-meter and Crison Basic 30 conductivity-meter, respectively). Then, the concentrations of water-soluble organic carbon (WSOC), water-soluble salts (Ws-$Cl^-$, Ws-$SO_4^{2-}$, Ws-$K^+$, Ws-$Na^+$, Ws-$Ca^{2+}$ and Ws-$Mg^{2+}$), water-soluble ammonium (Ws-$NH_4^+$, as an indicator of N mineralization) and water-soluble metal(loid)s (Ws-As, Ws-Cd, Ws-Cu, Ws-Pb and Ws-Zn) were analyzed. WSOC was analyzed in a total organic carbon analyzer (TOC-VCSH Shimadzu), soluble salts and ammonium by ion chromatography (Metrohm) and metal(loid)s by inductively coupled plasma mass spectrometry (ICP-MS Agilent 7500A; detection limit 0.002 mg $L^{-1}$).

Other thawed soil aliquots were used to evaluate microbial biomass carbon and the activity of the enzyme dehydrogenase and β-glucosidase (Kandeler, 2007). Microbial biomass carbon (MBC) was estimated following the fumigation-extraction method (Vance et al., 1987; Wu et al., 1990). Samples were fumigated with $CHCl_3$, incubated for 24 h at room temperature and organic C extracted with 0.5 M $K_2SO_4$ and measured with a total organic carbon analyzer (TOC-VCSH Shimadzu). Dehydrogenase activity (DH) was determined according to García et al. (1997). Samples were incubated with iodonitrotetrazolium chloride at room temperature for 20 h and the iodonitrotetrazolium formazan (INTF) formed was measured by spectrophotometry at 490 nm (Thermo Fisher Scientifc Multiskan GO). β-glucosidase activity (β-glu) was determined according to the modification of Ravit et al. (2003) proposed by Reboreda and Caçador (2008). Samples were incubated with *p*-nitrophenyl-b-D-glucopyranoside at 37 °C for 60 min and the released p-nitrophenol (pNF) was measured by spectrophotometry at 410 nm (Thermo Fisher Scientifc Multiskan GO).

### 2.3. In situ seasonal field monitoring

Organic matter decomposition, soil temperature and feeding activity of soil dwelling organisms were seasonally measured (Fig. S4, Suppl. Mat.). Organic matter decomposition was evaluated by the tea bag index (TBI) method (Keuskamp et al., 2013), with Lipton green Sencha exclusive collection tea (EAN: 8714100 77,054 2) and Lipton rooibos tea (EAN: 8222700 18,843 8). Tea bags ($n = 10$ per tea type and plot of study environment) were seasonally buried at ~10 cm depth and regularly collected to determine the remaining mass (after removing soil particles and drying at 70 °C for 48 h). Then TBI was calculated (http://www.teatime4science.org/publications/). Soil temperature was measured by inserting digital thermometers at ~5 cm depth at each tea bags sampling time ($n = 3$ per plot of study environment). Bait-lamina sticks (*Terra Protecta*® GmbH, Berlin, Germany) were used to estimate the feeding activity of soil dwelling organisms (Kratz, 1998; ISO, 2016). Two groups of 5 baited sticks ($n = 10$ per plot of study environment) 10 cm long were seasonally buried. Each stick had 16 holes filled with a mixture of cellulose (70%) and bran powder (30%) together with a small amount of activated carbon. After 20 d the number of holes partially and fully empty was recorded. Several studies have shown that soil macrofauna, mesofauna and microarthropods are the main feeders of bait lamina, while microorganisms do not contribute to perforate the baits during short periods (*i.e.*, 15–20 d) (Boshoff et al., 2014 and references cited therein).

### 2.4. Statistical analyses

Statistical analyses were performed with IBM SPSS Statistics 19.0.0 (SPSS, 2010). Differences were considered significant at $p < 0.05$. Data were transformed when necessary to achieve normal distribution (Shapiro-Wilk's test) and/or homogeneity of variances (Levene's test). Different transformations were used, depending on the nature of the data, including logarithmic- and arcsine square root-transformations. Repeated-measures ANOVA (RM-ANOVA), followed by Bonferroni post-hoc test, was used to compare the seasonal changes in the measured parameters and the differences among the study environments. The factors included in the analysis were: 1) intra-subject factor, the season -repeated factor- (summer, autumn, winter, and spring); 2) inter-subject factor, the environment (B, P, P + S, DP + S, FN, and FA). A significant effect of the season indicated that the parameter evolved significantly over the study seasons. A significant effect of the environment indicated that the average parameter value differed among the study environments. A significant effect of season × environment interaction indicated that the evolution of the parameter over the study seasons differed among the study environments. For the specific case of soil moisture and temperature, RM-ANOVA was performed separately for each season, considering the sampling day as the intra-subject factor. Spearman's correlations were calculated to evaluate the relationships among all the assessed parameters. A Principal Component Analysis (PCA) per study season was applied to identify gradients relative to the parameters measured by using the 'CANOCO for Windows' program v4.02 (Jogman et al., 1987; ter Braak and Smilauer, 1999). The analyses were centered and standardized by environmental variables according to Jogman et al. (1987) and ter Braak and Smilauer (1999).

## 3. Results

### 3.1. Soil moisture and temperature

Total precipitation during the four seasonal sampling campaigns varied between ~4 mm (summer) and ~ 88 mm (winter) (Fig. S5, Suppl. Mat.). Weekly average air temperature was always >9 °C, with warmer conditions in summer (average 24.0 °C) and colder in winter (average 12.7 °C) (Fig. S5, Suppl. Mat.).

Variations in soil moisture content and temperature within each season (Fig. 1) were always affected by the sampling day, the environment, and the interaction of both factors (significant, $p \leq 0.019$; Table S2, Suppl. Mat.). Throughout summer, soil moisture progressively decreased from ~6–9% to ~3–4% in all the environments (Fig. 1a), except in P (always <2%, significantly lower). On the contrary, soil temperature progressively increased from ~19–20 °C to ~24–26 °C (Fig. 1a), except in B that reached significant higher values (~30 °C). Throughout autumn, soil moisture (Fig. 1b) oscillated from ~4–6% to ~14–17% in P + S, DP + S, FN and FA and was always <10% in B and P (significantly lower). During this period, soil temperature (Fig. 1b) progressively declined from ~22–24 °C to ~12–14 °C in B, FN and FA and to ~8–10 °C in P, P + S and DP + S (significant differences between both groups). Throughout winter, P was significantly

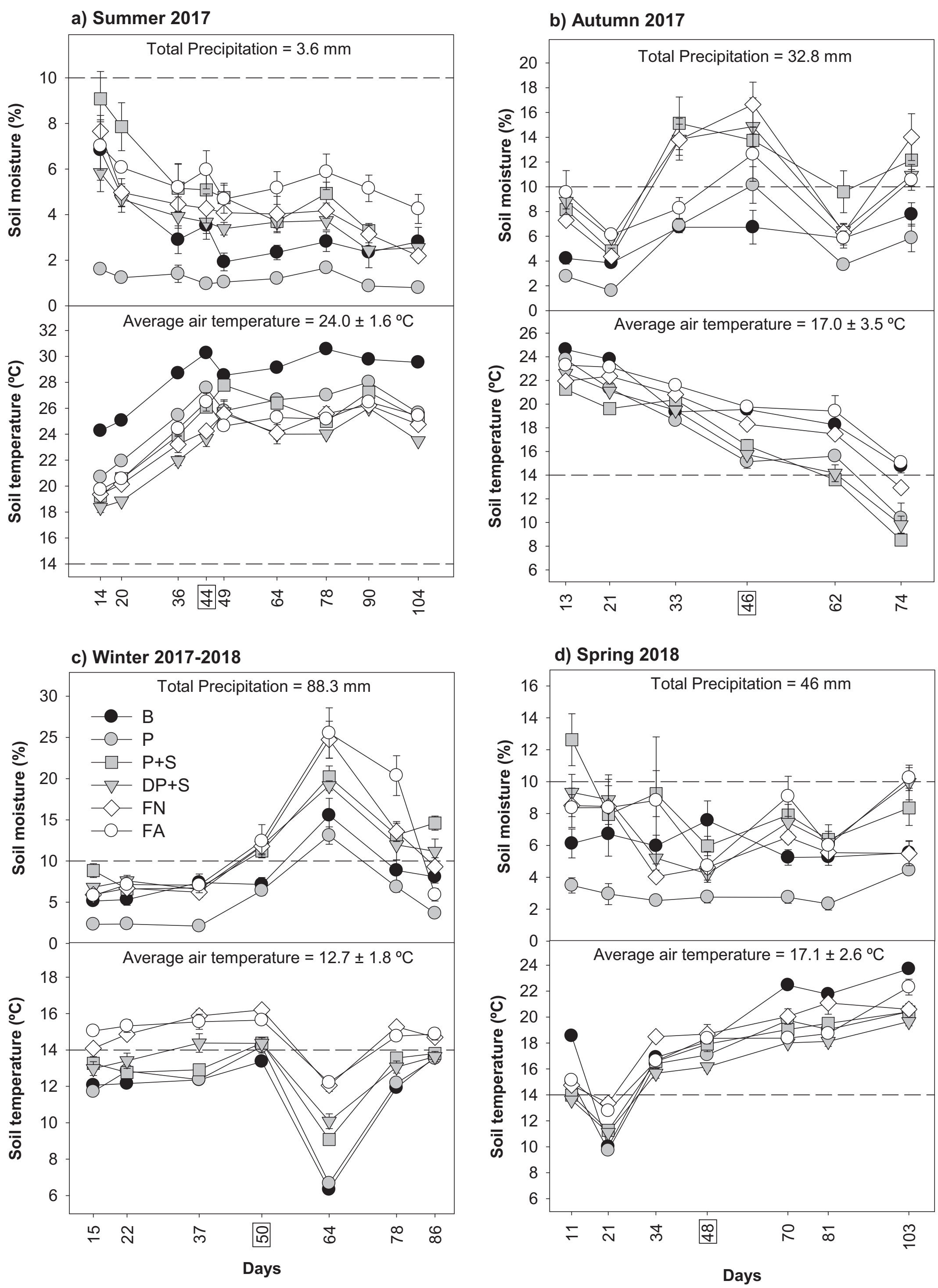


**Fig. 1.** Changes in soil moisture and temperature conditions in the study environments throughout each seasonal sampling campaign: summer (a), autumn (b), winter (c), and spring (d). Values are average ± SE ($n = 4$). Total precipitation and average air temperature refer to total precipitation and average air temperature during each sampling period. Horizontal dashed lines are drawn as reference to facilitate comparisons among study seasons. The day within a box in the X-axis indicates the day when soil samples were collected within each season for chemical and biological parameters determination. See the text for meaning of abbreviations of each environment.

drier than the other study environments (~2–13% vs. ~5–26%) and three groups were segregated regarding soil temperature (Fig. 1c): B and P were colder (~6–14 °C), FN and FA warmer (~12–16 °C), and P + S and DP + S showed intermediate values (~9–14 °C). Finally, throughout spring, soil moisture was ~2–4% in P (significantly lower) and varied, most of the time, between ~5% and ~ 9% in the other environments (Fig. 1d). During this last season soil temperature ranged from ~11–14 °C to ~19–23 °C, with scarce differences among environments (Fig. 1d).

### 3.2. Soil chemical parameters (pH, EC, water-soluble salts and metal(loid)s)

Soil pH was significantly lower in B than in the other study environments (~5.5–6.0 vs. ~7.2–8.3; effect of environment, $p = 0.000$) (Fig. 2a and b; Table S3, Suppl. Mat.). It was also affected by the season, increasing in autumn, and the season x environment interaction (significant seasonal changes in FA) ($p = 0.000$; Table S3, Suppl. Mat.). Soil EC was significantly affected by the environment and the season x environment interaction ($p \leq 0.010$; Table S3, Suppl. Mat.). The most saline soils were found in B (EC ~4–7 d $Sm^{-1}$) (Fig. 2c), with significant higher concentrations of Ws-$Cl^-$, Ws-$SO_4^{2-}$, Ws-$Na^+$ and Ws-$Mg^{2+}$, which increased during summer and spring (Fig. S6, Suppl. Mat.). The less saline soils were found in FN and FA (EC <1 dS $m^{-1}$) (Figs. 2d). The last environment, in turn, showed the greatest concentration of Ws-$K^+$ and high levels of Ws-$Cl^-$ and Ws-$Na^+$ (Fig. S6, Suppl. Mat.).

The behavior of water-soluble metal(loid)s differed depending on the element considered (Fig. 3), but the concentrations were always significantly affected by the environment and the season x environment interaction ($p \leq 0.035$; Table S3, Suppl. Mat.). Ws-Mn, Ws-Pb and Ws-Zn were significantly higher in B (~6700–11,500 μg $kg^{-1}$, ~1900–3800 μg $kg^{-1}$ and ~ 104,000–207,000 μg $kg^{-1}$, respectively), while FA showed significant higher concentrations of Ws-As (~90–190 μg $kg^{-1}$). A tendency to increasing concentrations in summer and spring was observed for Ws-Mn in B and P + S, for Ws-Pb in FA, for Ws-Zn in P and P + S and for Ws-As in FN and FA.

### 3.3. Soil biological parameters (WSOC, MBC, Ws-$NH_4^+$, DH, β-glu, TBI and feeding activity)

The season, the environment and the season x environment interaction significantly affected WSOC, MBC, Ws-$NH_4^+$, DH and β-glu ($p \leq 0.035$; Table S3, Suppl. Mat.). The environment B (Fig. 4) showed the lowest values of WSOC (~12–19 mg $kg^{-1}$), MBC (~5–18 mg C $kg^{-1}$) and DH (~0.02–0.19 μg INTF $g^{-1}$ $h^{-1}$); β-glu was not detected. By contrast, the highest values of WSOC, MBC and Ws-$NH_4^+$ were found in FA (with greater scores in summer and spring), followed by FN (with a tendency to increasing scores towards spring): WSOC ~490–1420 mg $kg^{-1}$ in FA and ~ 170–370 mg $kg^{-1}$ in FN; MBC ~1040–1520 mg C $kg^{-1}$ in FA and ~ 300–600 mg C $kg^{-1}$ in FN; Ws-$NH_4^+$ ~ 7.4–8.9 mg $kg^{-1}$ in FA and ~ 3.0–8.6 mg $kg^{-1}$ in FN (Fig. 4). The vegetated tailing environments (P, P + S and DP + S) showed intermediate values, especially for WSOC and MBC, which also tended to increase towards spring. The environment P had the highest DH activity (~4.7–8.4 μg INTF $g^{-1}$ $h^{-1}$), which increased towards spring despite belonging to a tailing area, while FN and FA showed the highest values of β-glu activity (~1.9–3.8 μmol PNF $g^{-1}$ $h^{-1}$) (Fig. 4f and g).

Organic matter decomposition (evaluated by the TBI method) was affected by the season, the environment, and the combination of both factors ($p \leq 0.013$; Table S3, Suppl. Mat.). A progressive increase in TBI from summer to spring was observed in all the study environments (from ~0.001–0.006 to ~0.007–0.008) (Fig. 4h). The feeding activity of soil dwelling organisms was detected in all the environments in summer, autumn and spring, with large variability and without clear differences among them (Fig. S7, Suppl. Mat.). In winter, higher activity was clearly observed in forest soils outside the mine tailings (0–4 total fed holes in B, P, P + S and DP + S vs. 30–45 total fed holes in FN and FA).

### 3.4. Environmental gradient analysis

The PCAs performed separately for summer, autumn, winter, and spring (73.7%, 82.0%, 77.1% and 69.8% of variance explained by the two first

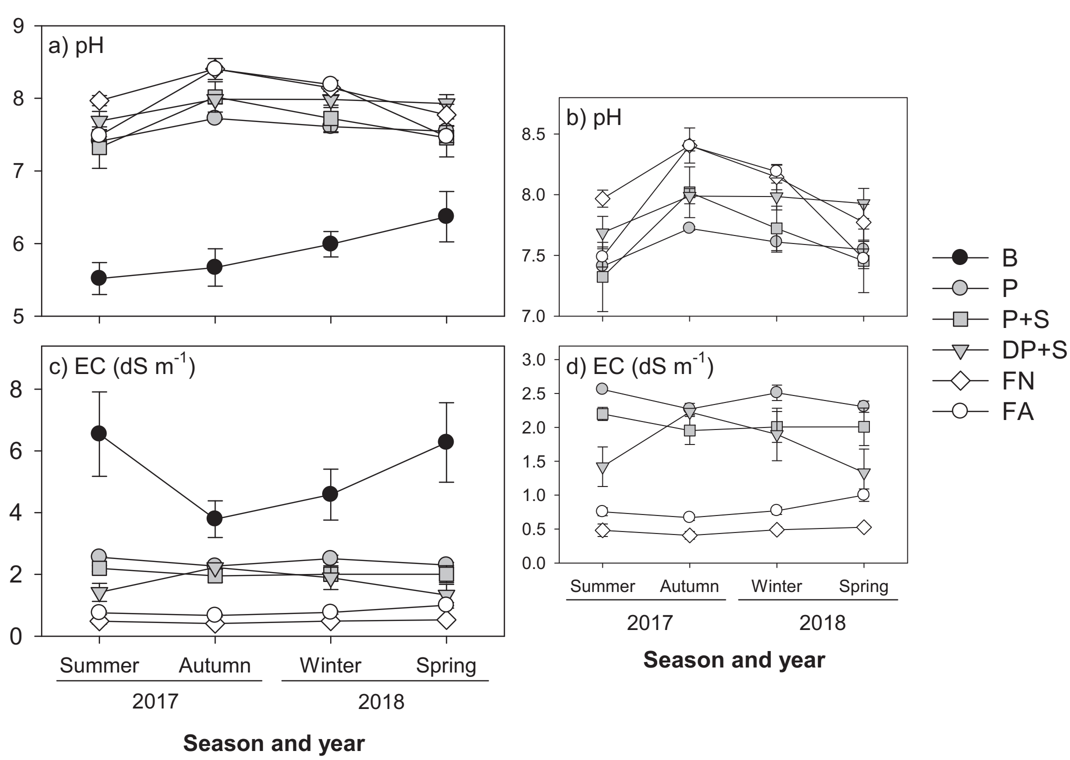


**Fig. 2.** Seasonal changes in soil pH (a and b; b is a zoom of a) and electrical conductivity -EC- (c and d; d is a zoom of c) in the study environments. Values are average ± SE (n = 4). See the text for meaning of abbreviations of each environment.

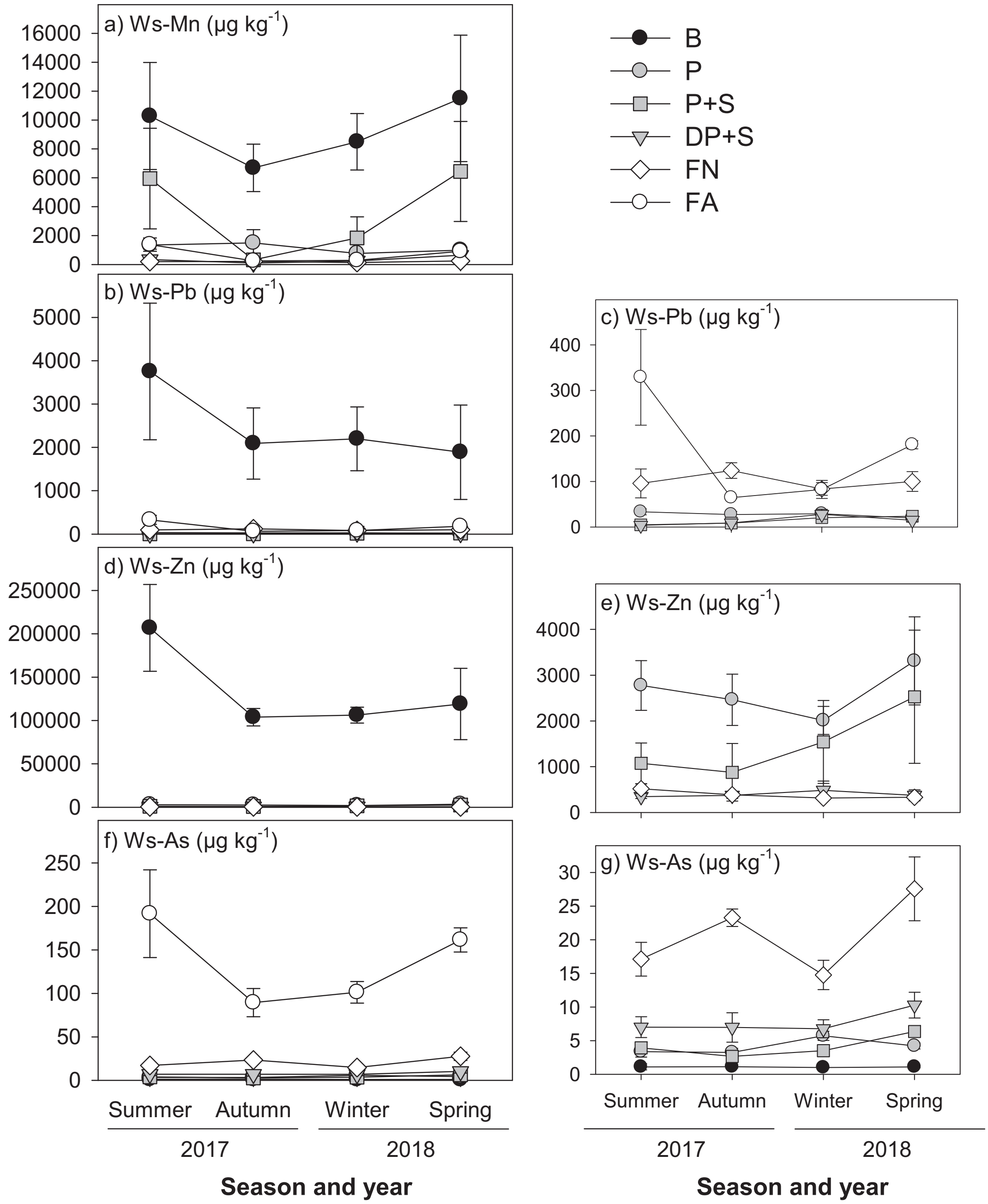


**Fig. 3.** Seasonal changes in water-soluble metal(loid) concentrations in the study environments: Ws-Mn (a), Ws-Pb (b and c; c is a zoom of b), Ws-Zn (d and e; e is a zoom of d), and Ws-As (f and g; g is a zoom of f). Values are average ± SE (n = 4). See the text for meaning of abbreviations of each environment.

axes, respectively) showed the different contribution of the parameters studied to the gradients throughout seasons (Fig. 5).

Soil quality and functional parameters characterized the primary gradient (X-axis) regardless of the study season, but the influence of the climatic factors changed. The plots from the B environment (bare soils) were depicted on the positive side as sites with more detrimental conditions, as shown by the higher acidity and salinity (i.e., lowest pH and highest EC) and greater concentrations of Ws-Mn, Ws-Pb and Ws-Zn. By contrast, FN and FA plots (forest soils) were depicted on the negative side as sites with higher scores for biological parameters (WSOC, MBC, Ws-$NH_4^+$ and DH), but also with higher Ws-As. The vegetated environments within the mine tailings (P, P + S and DP + S) were located at intermediate positions between the forests and bare soils.

Soil moisture content was more relevant for the primary gradient in autumn and winter (Fig. 5b and c), contributing to segregate FN and FA towards the negative side as the wettest sites and B towards the positive side as the driest sites. By contrast, soil moisture played a minor role in summer and spring when the arrows of this variable were depicted at intermediate positions between the X and Y axes (Fig. 5a and d). Soil temperature was related to the primary gradient in summer (with FN and FA as the coldest sites and B as the warmest ones) and winter (with FN and FA as the warmest sites and B as the coldest ones), but in autumn and spring it was a relevant variable for the secondary gradient (Fig. 5b and d).

## 4. Discussion

We evaluated climatic, chemical, and biological soil conditions in abandoned metal(loid) mine tailings from a semiarid area to assess whether vegetation-induced soil improvement was consistent and, to what degree, modulated throughout seasons. Previous studies already showed that

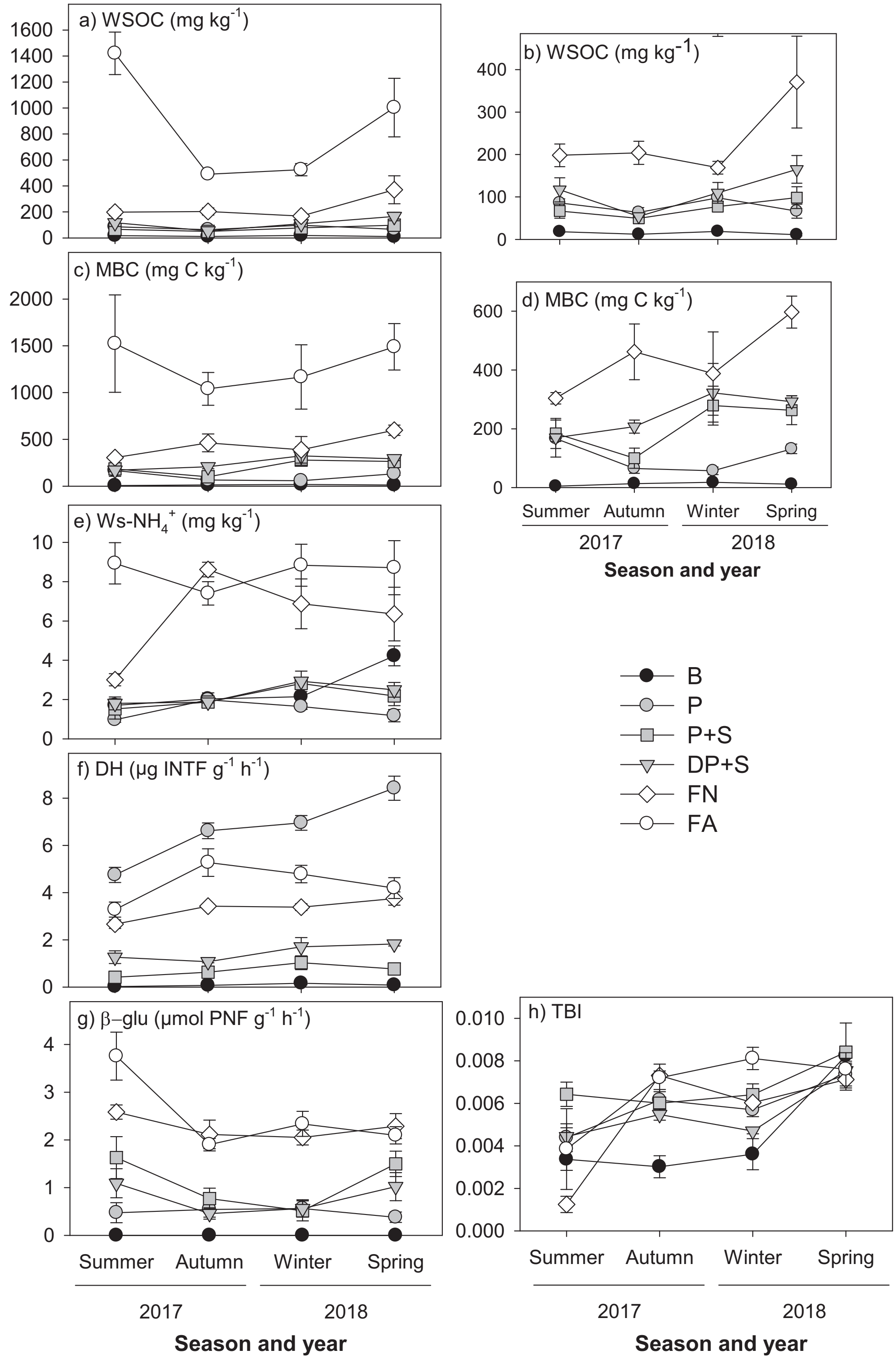


**Fig. 4.** Seasonal changes in soil biological parameters in the study environments: WSOC, water-soluble organic carbon (a and b; b is a zoom of a); MBC, microbial biomass carbon (c and d; d is a zoom of c); Ws-$NH_4^+$, water-soluble ammonium (e); DH, dehydrogenase activity (f); β-glu, β-glucosidase activity (g); TBI, tea bag index (h). Values are average ± SE (n = 4). See the text for meaning of abbreviations of each environment. INTF (iodonitrotetrazolium formazan). PNF (p-nitrophenol).

mine tailings soils beneath vegetation patches improve relative to bare areas (Párraga-Aguado et al., 2013), reaching a functional state similar to that of forest soils outside tailings (Álvarez-Rogel et al., 2021; Peñalver-Alcalá et al., 2021). However, these studies did not consider seasonal variability. Overall, our results indicated that soil improvement in vegetated patches within mine tailings was robust throughout the year (Figs. 2-4), and contributed to locating P, P + S and DP + S nearer to the forests (FN and FA) than to bare (B) soils in the environmental gradients defined by the PCAs (Fig. 5). In turn, soil moisture content and temperature suffered remarkable variations both among and within seasons (Fig. 1), which was reflected in their different seasonal contribution to the environmental gradients (Fig. 5). Since we worked in two mine tailings, it is plausible that the micro-climatic/topographic conditions were not the same when they were abandoned ~40 years ago. However, it is reasonable to assume that

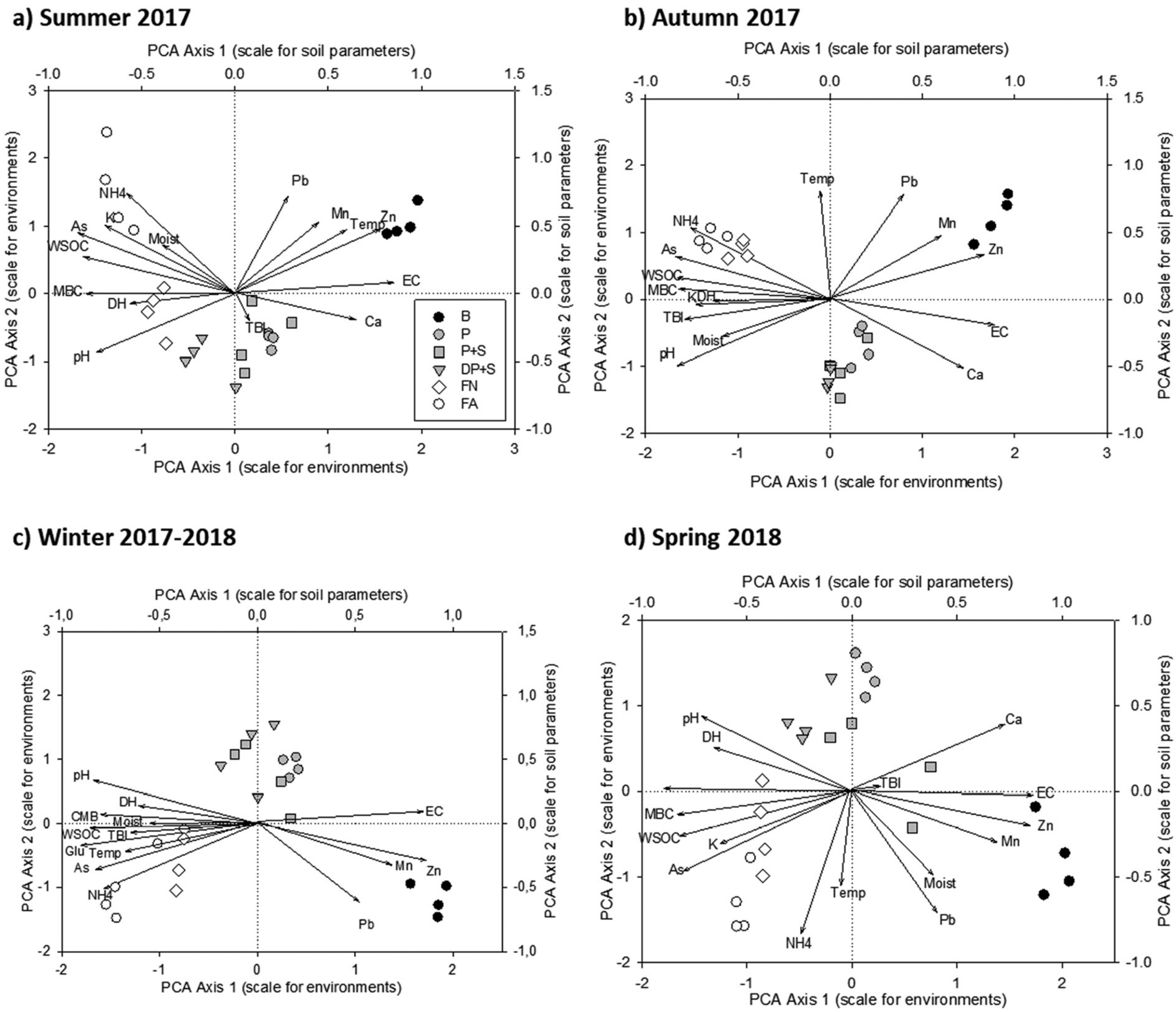


**Fig. 5.** Results of the seasonal Principal Component Analyses (PCAs) in the study environments. Cumulative percentage of variance explained by the first two axes: Summer 73.7%; Autumn 82.0%; Winter 77.1%; Spring 69.8%. EC: electrical conductivity; WSOC: water-soluble organic carbon; MBC: microbial biomass carbon; DH: dehydrogenase activity; Glu: β-glucosidase activity; TBI: tea bag index; Temp: soil temperature; Moist: soil moisture. Ca, K, Mn, Zn, Pb, As, and NH4 are water-soluble concentrations (1:2.5 extracts). See the text for meaning of abbreviations of each environment.

the time elapsed has been enough to depict, in a realistic way, the changes induced by spontaneous plant colonization in tailing soils and the contribution of climatic, chemical, and biological soil conditions to the environmental gradients identified.

### *4.1. Variability of soil climatic conditions*

The facilitative effect of vegetation to ameliorate abiotic soil stress conditions by softening radiation and temperature has been stated by other authors (*e.g.*, Callaway et al., 2002; Navarro-Cano et al., 2019). In the case of semiarid mine tailings soils, the milder microclimatic conditions below the patch canopy have been considered even more relevant to trigger biota succession than the induced improvement in edaphic characteristics (Navarro-Cano et al., 2019; Colin et al., 2019; Oreja et al., 2020). The cited authors, however, did not analyze factors such as water-soluble salts and metal(loid)s, which are considered key for plant establishment in these hostile environments (Párraga-Aguado et al., 2013, Parraga-Aguado et al., 2014a). Our results indicate that the relevance of soil moisture content and temperature was seasonally modulated, while the role of soil chemical and biological conditions was quite stable (Fig. 5). For instance, in summer, bare soils (B) directly exposed to insolation reached higher temperatures than vegetated environments, while, in winter, it was one of the environments in which soil temperature dropped the most (Figs. 1 and 5). In turn, in P environment, the driest soils were found in summer and spring, but differences with other environments were reduced in autumn and winter (Fig. 1). The forest soils (FN and FA) tended to be colder in summer and warmer and wetter in winter, but these differences vanished in the other seasons. All this indicates that soil moisture content and temperature were influenced not only by the air temperature and precipitation conditions of the study area, but also by the edaphic characteristics of the mine tailings soils and the vegetation cover.

D'Odorico et al. (2007) discussed soil moisture-vegetation feedbacks in vegetated patches and adjacent gaps of an arid savanna. They found that when surface soils were wet, they were wetter under canopies, and when surface soils were dry, they were drier under canopies. We did not find this pattern when comparing soils from bare areas (B) with those from vegetated patches in any of the seasons (Fig. 1), probably due to the influence of properties such as soil texture and structure in water retention capacity and the presence of plant species with distinct behavior in relation with water uptake in the study environments (Álvarez-Rogel et al., 2021; Peñalver-Alcalá et al., 2021). It is expected that soils with pedogenetic structure (such as P + S, DP + S, FN and FA) retain more water than unstructured soils (such as B). Also, vegetation contributes to maintain wetter soils due to the shadow effect and the capacity of the canopy to intercept rainfall and supply water by the stem flow effect that funnels rainwater down to the soil (D'Odorico et al., 2007). The driest soils in P environment could be explained by its sandier texture (Table S1, Suppl. Mat.), attributable to the accumulation of wind-blown sand particles around pine trunks (Álvarez-Rogel et al., 2021), and this could have counterbalanced the favorable effect of vegetation to favor wetter soil conditions.

Factors affecting soil temperature include organic matter content, color, surface mulching, plant cover, radiation, and evaporation (Onwuka and Mang, 2018 and references cited therein). In the forest soils outside the mine tailings (FN and FA), the higher content of organic matter contributed to a darker color (Álvarez-Rogel et al., 2021), which could have favored greater heat retention in the soil (Fang et al., 2005). The latter was more evident in winter, the season with the lowest air temperature values (Fig. S5, Suppl. Mat.). Furthermore, during winter, FN and FA tended to be wetter, which could also decrease soil heat dissipation (Ochsner et al., 2001). In turn, P + S and DP + S, with higher organic matter content and darker color than B and P (Álvarez-Rogel et al., 2021), showed an intermediate behavior, especially during winter, with less accentuated changes in soil temperature (they did not reach temperatures as low as B and P) (Fig. 1).

### *4.2. Relationship among climatic and chemical and biological soil conditions*

The significant effect of the factors season and environment and of the interaction of both for most of the edaphic parameters measured (Table S3, Suppl. Mat.) indicates that the climatic factors temperature and moisture affected soil conditions in a different way in the study environments depending on the period of the year.

Extreme soil temperatures and dryness are typically reached during warmer periods in semiarid areas. Thus, if salts are present, soil salinity increases in the upper layers due to the capillary upward movement of soluble ions in the soil profile (González-Alcaraz et al., 2014). These features that contributed to characterize environment B in summer (Fig. 5), where soil temperature reached values up to ~30 °C (Fig. 1) and EC and most of the water-soluble salts increased by 1.5–2-fold (Fig. 2; Fig. S6, Suppl. Mat.), are co-occurring factors that hinder plant colonization. This highlights the value of introducing adapted xerophyte local plant species, including halophytes, for the phytomanagement of bare mine tailings in semiarid areas (Parraga-Aguado et al., 2014). Furthermore, the selected plant species should also be able to immobilize metal(loid)s in the rhizosphere, with low translocation rates to aerial parts, to restrict the transfer of PTEs to the food web (Robinson et al., 2009). Unlike bare soils (B), soil salinity variability was less pronounced in vegetated patches within mine tailings (P, P + S and DP + S) and did not follow a clear seasonal pattern (Fig. 2). This could be attributable to the modulation effect of vegetation for softening extreme increases in soil temperature during the warmer seasons. Contrary to EC, seasonal changes in pH were more evident in vegetated environments, with a tendency to increase in autumn and winter and decrease in summer and spring (Fig. 2). In fact, a significant positive correlation was found between soil pH and moisture ($r = 0.336$, $p = 0.001$). This relationship was more evident in the forest away (FA), the environment with higher scores for soil biological parameters (Fig. 5). A decrease in pH is expected when biological activity increases. This is particularly obvious in the rhizosphere where microbial processes, such as organic matter mineralization (that releases $CO_2$) and nitrification (that produces $H^+$), are more intense and roots activity release $H^+$ and organic acids (Hinsinger et al., 2009).

A tendency to higher water-soluble metal(loid) concentrations in summer and spring was observed (Fig. 3). Correlations performed with the whole data set did not shed any light on the possible relationship between climatic soil factors (temperature and moisture) and water-soluble metal (loid)s. To remove a possible bias due to the influence of the acidic pH of bare soils (B), new correlations were made excluding these data. The results obtained showed significant positive correlation between soil temperature and Ws-As ($r = 0.241$, $p = 0.031$) and significant negative correlations between soil moisture and Ws-Mn and Ws-Zn ($r \geq -0.265$, $p \leq 0.018$). Although dissolved metal(loid)s can be transported through soil pores and cracks like soluble salts, such mobility is restricted since their solubility highly depends on the geochemistry of each element in relation to the pH/Eh system and the presence of specific soil components (Simón et al., 2010, 2015). The largely higher concentration of Ws-Mn, Ws-Pb and Ws-Zn in bare soils (B) were significantly correlated with the low pH of this environment ($r \leq -0.372$, $p = 0.000$) since the solubility of these metals increases under acidic conditions. In the study environments with neutral-basic pH, the concentrations of Ws-Mn and Ws-Zn consistently decreased from P to FA, regardless of the total metal content. This could be related with the increasing total $CaCO_3$ content from P to FA (Table S1, Suppl. Mat.) (Simón et al., 2010). However, Ws-Pb concentrations were higher in FN and FA than in P, P + S and DP + S, despite the high T-Pb levels found in tailing soils (Table S1, Suppl. Mat.). The latter could be related to the greater efficiency of Fe-oxy-hydroxides, which are more abundant in tailings soils (Table S1, Suppl. Mat.; Parraga-Aguado et al., 2015), to control Pb dissolution than carbonates (Palumbo-Roe et al., 2009; Simón et al., 2010). Contrary to the other elements, Ws-As concentrations were higher in FN and FA (Fig. 3) and significant positive correlations were found between Ws-As and WSOC and pH ($r \geq 0.540$, $p = 0.000$). Our results agree with previous findings that showed an increase of As mobility when labile organic matter concentration increases (Simón et al., 2015). Moreover, under high pH and in the presence of $CaCO_3$ the predominant species is $HAsO_4^{2-}$, which displays low adsorption capacity on solid surfaces and low co-precipitation with Fe-oxides (González et al., 2012; Simón et al., 2015), facilitating the presence of Ws-As.

No significant correlations were found between climatic and biological soil parameters when considering the whole data set. However, when correlations were performed without the B environment, significant positive correlations were found between soil temperature and WSOC, MBC and β-glu activity ($r \geq 0.259$, $p \leq 0.021$). The latter supports that soil microbial activity was stimulated in warmer periods. Soil respiration (Salazar et al., 2011; Meena et al., 2020), enzyme activity (Boerner et al., 2005; Hedo et al., 2015; Zuccarini et al., 2020) and MBC (Feng et al., 2009) have shown seasonal variations in a variety of ecosystems, but without clear consistent patterns. Rising temperatures can increase microbial activity and thus organic matter processing and turnover (Sofi et al., 2016). Also, in semiarid areas, decomposition is typically accelerated if moisture is adequate (Insam, 1990). Tomar and Baishya (2020) found positive correlations between soil respiration, MBC and DH activity with the increase in soil moisture content after a dry season and stated that seasonality played a significant role in determining variations in soil microbiological processes. Bastida et al. (2008) found an increase in WSOC in spring and autumn relative to summer in semiarid SE Spain, but this was modulated by the vegetation type and slope orientation. Other factors such as plant physiological activity that controls root exudates and litter production, which are seasonally modulated, also influence soil microbial activity (Eslaminejad et al., 2020). Our results showed spatial (among environments) and temporal (among seasons within environments) variability in edaphic conditions, but the improvement of soil microbiology beneath vegetation patches was obvious and consistent throughout seasons, which demonstrates the interest to preserve vegetation patches spontaneously formed in abandoned mine tailings.

Regarding the feeding activity of soil dwelling organisms, we found eaten baits in most of the study environments during the four seasons (Fig. S7, Suppl. Mat.). Previous studies have found variable, and even contradictory, results of feeding activity determined by bait lamina sticks. Differently to Simpson et al. (2012), who showed that the feeding activity of soil organisms was compromised under drought stress, we did not find lower activity in summer. Within the mine tailings, we found a tendency to higher feeding activity in vegetated patches than in bare soils, but the results were highly variable and without clear patterns among environments and seasons. Feeding activity is considered of paramount importance for the maintenance of soil biogeochemical cycles (and hence soil function), since the rate of organic matter decay and nutrients recycling primary depends on micro and mesofauna activity (Morgado et al., 2018). Many environmental factors influence fauna activity, including vegetation characteristics, soil organic matter content and type, moisture and temperature and soil pollution (Boshoff et al., 2014 and references cites therein). The existence of such a variety of co-occurring factors makes it difficult to identify the main drivers of activity under field conditions. Although previous works have stablished the deleterious effects of high metal(loid) levels in the activity of soil invertebrates (Filzek et al., 2004; Boshoff et al., 2014), we found evidence of feeding even in mine tailing soils with extremely high PTE concentrations. Moreover, our data point out the value of vegetated patches spontaneously formed for harboring this activity. Also, our results, in general, agree with previous evidence that showed similar, or even less, eaten baits in good quality forest soils with large amounts of organic remains than in degraded polluted soils (André et al., 2009). This could be attributable to the preference of soil organisms for native debris, abundant in the first soils, while in the second ones the scarcity of local resources could favor baits consumption. Moreover, invertebrates could have been attracted to the baits buried in the bare soils (B) in their search for food and moved from the vegetated patches to consume them.

Winter was the only season when the number of eaten baits per stick were clearly higher in the forest soils outside the mine tailings (FN and FA) (although some activity was also detected in P + S and DP + S) (Fig. 5), which demonstrates the importance of considering seasonality when studying invertebrates' feeding activity. Gongalsky et al. (2008) induced higher feeding activity (measured by bait lamina) in 15-day incubation experiments when temperature was increased from 14 °C to 24 °C, while moisture played a minor role. André et al. (2009) found more baits eaten in winter in warmer sites with temperatures reaching ~12 °C. Our findings agree with the authors cited, since, in winter, forest soils (FN and FA) and, in less extent, vegetated patches with plants growing beneath pine trees canopy (P + S and DP + S), maintained soil temperature > ~14 °C most of the time. This highlights the key role of vegetation not only to provide organic matter and nutrients, but also to regulate soil climate conditions and so facilitate biological activity.

## 5. Conclusions

The improvement in climatic, chemical and biological soil parameters induced by vegetation spontaneously colonizing metal(loid) mine tailings in semiarid areas was seasonally consistent. This was more evident for those vegetated patches with herbs and shrubs under the tree canopy (P + S and DP + S) that showed better edaphic conditions than bare soils (B) and the patches only composed by trees (P) throughout the study period, and closer to the forest soils outside the tailings (FN and FA). Soil temperature and moisture changed over seasons, and this was modulated by the characteristics of the tailing soils (mainly structure, texture, and organic matter content) and the plant cover. The environments devoid of vegetation (B) or with a simpler soil-vegetation system (P) did not counteract climatic variations and reached extreme values of soil temperature and moisture during the most contrasting seasons (summer and winter). However, P + S and DP + S were able to soften the harsh climate conditions of summer and winter, more like FN and FA. The most extreme temperature and dryness conditions reached during summer in B were related with sharp increases in electrical conductivity (EC), and thus water-soluble salts, and water-soluble metals. These variations were buffered in P + S, DP + S, FN, and FA. On the contrary, the changes in biological and biological-related parameters were in general more pronounced in vegetated patches and surrounding forests in relation to its greater biological activity.

Our findings reinforce the idea of preserving spontaneously formed vegetation patches as part of the phytomanagement strategies of abandoned metal(loid) mine tailings in semiarid areas. These vegetation spots might act as key ecosystem structures that contribute to plant recruitment, accelerating the recovery, and confer greater functionality and, therefore, greater sustainability and resilience against future climate stresses. In addition, phytomanagement practices should select a mix of plant species with the capacity to create patches of vegetation with high diversity. This enhances the ability to smooth drastic climatic changes and provides a wide variety of resources to facilitate soil biological activity.

## CRediT authorship contribution statement

**José Álvarez-Rogel**: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – Original Draft, Writing - Review & Editing, Supervision, Project administration, and Funding acquisition.

**Antonio Peñalver-Alcalá:** Methodology, Investigation, Data curation, and Writing - Review & Editing.

**M. Nazaret González-Alcaraz**: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration, and Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.155945.

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